THE REMARKS

Claims 1, 4, 5, 7-9, 13-16, 19, and 21-27 were pending prior to entering the amendments.

The Amendments

Claim 1 is amended to clarify the meaning of the claim. The amendment of Claim 1 is supported by Publication 2005-0095605 at Paragraphs [0014], [108], [109], [0154], [158] and Figure 3.

The amendments of FIGURES 3-2 and 3-3 are to insert the bold "H". A marked-up copy is attached herewith to show the changes made. At Paragraph [0098], the application describes that FIGURES 3-1 to 3-8 depict an alignment of the amino acid sequences of 81 natural proteorhodopsin variants. The bold "**H**" indicates the position of a conserved histidine, which corresponds to H75 of Bac31A8. Applicants inadvertently omitted the labeling of "H" in FIGURES 3-2 to 3-3 as filed, and are submitting herewith replacement FIGURES 3-1 to 3-8 to insert the bold "**H**". Support for the amendment can be found in Figure 2-2 of the Provisional Application No. 60/429,518, which is incorporated by reference under § 1.57(a). Support for the amendment can also be found in Paragraph [0098] of the instant application, where it describes that the bold "**H**" corresponds to H75 of Bac31A8. In Figures 3-1, because of the 6-amino acid gap in the alignment of BAC31A8, the conserved H is shown at position 81, which is the 75th amino acid.

No new matter is added in the amendments. The Examiner is requested to enter the amendments.

Restriction Requirement

The key of this invention is that Applicant has discovered that a mutation in the conserved histidine of a naturally occurring proteorhodopsin provides a proteorhodopsin mutant with improved optical characteristics, i.e. has lower pK_{rh} in comparision with the naturally occurring proteorhodopsin. Such technical feature was not taught or disclosed in any prior art.

The Examiner found Applicants' arguments unpersuasive because the phrase "conserved histidine" was unclear. Applicants have amended Claim 1 to clarify the meaning of the claim. In

view of the claim amendment and the filing of RCE, Applicants respectfully request that the Examiner reconsiders the restriction requirement.

35 U.S.C. 112, Second Paragraph Rejection

Claims 1, 4-5, 7-9, 14, 23-27 remain rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The rejection is overcome in view of the claim amendment.

Conserved histidine residue

The Examiner states that the phrase "conserved histidine residue" in claim 1 (and its dependent claims 4-5, 7-8, 14) is unclear. Applicants have amended the claim to recite that the conserved histidine is present at the position equivalent to position 75 of SEQ ID NO: 3 when the proteorhodopsin variant is aligned with SEQ ID NO: 3 for a maximum identity.

At Paragraph [0014], the application describes that a conserved histidine residue is at, for example, amino acid position 75 of Bac31A8, or position 77 of Hot75m1, or its equivalent position of a proteorhodopsin variant.

At Paragraph [0154], the application describes methods known in the art for aligning protein sequences. There can be deletions or insertions when two structures are aligned for maximum identity. For example, proteorhodopsin Bac31A8 has only 249 amino acids while proteorhodopsin Hot75m1 has 252 amino acids. Aligning the two sequences shows that Bac31A8 has no residue corresponding to 214 of Hot75M1. Thus, the amino acid sequence of Bac31A8 would appear very different from Hot75m1 unless a gap is recorded between locations 211 and 212 of Bac31A8 (see FIG. 2 for alignment).

As shown in Figure 3, naturally occurring proteorhodopsins have high degree of homology. Applicants have shown "the conserved histidine" in 81 naturally occurring proteorhodopsins. **In the 81 proteorhodopsin sequences shown in Figures 3-1 to 3-8, 57 of them contain only one histidine residue, which is the conserved histidine residue.** The remaining 24 proteorhodopsins (HOT2C02, HOT75m3, medA15_R8_3, medA15_r8ex7, medA15_R8ex9, medA15_r9_3, medA15r10b5, medA15r11b3, medA15r8b3, medA15r8b8,

medA15r8b9, medA15r8ex4, medA15r9b5, medA15r9b7, medA17_r8_11, medA17_r8_15, medA17_R8_6, medA17R9_1, medA19_R8_16, medA19_R8_19, medA19_R8_20, medA19_r9_9, PalB1, and PalE6) contain two histidine residues.

There are only very few histidine residues in the naturally occurring proteorhodopsins, as illustrated in Figures 3-1 to 3-8. **A skilled person in the art can easily compare and align the amino acid sequences of Bac31A8 and any naturally occurring proteorhodopsin or its proteorhodopsin homolog having 90% identity and determine where the conserved histidine is.**

Therefore, the phrase "the conserved histidine" in a naturally occurring proteorhodopsin is not indefinite.

Having at least 90% identity

The Examiner states that the phrase "having at least 90% identity" in claim is unclear. Applicants have amended Claim 1 to recite the proteorhodopsin mutant is a proteorhodopsin variant comprising a mutation in a conserved histidine residue, said proteorhodopsin variant is a naturally occurring proteorhodopsin or a proteorhodopsin homolog having at least 90% identity with the naturally occurring proteorhodopsin.

Paragraph [0003] describes that proteorhodopsins are integral membrane proteins isolated from uncultivated marine eubacteria and function as light-driven proton pumps. The naturally occurring proteorhodopsin in Claim 1 is meant to include naturally occurring proteorhodopsins isolated from marine eubacteria.

Paragraph [0154] describes that closeness of relation can be measured by comparing amino-acid sequences. Methods of aligning protein sequences and methods of defining relatedness are described in the application and well known to a person skilled in the art. A skilled person can easily align a naturally occurring proteorhodopsin with its homolog and compare them for percent of identity.

Therefore, the phrase "having at least 90% identity" is not indefinite.

35 U.S.C. 112, First Paragraph Rejection

Claims 1, 4-5, 7-9, 14, 23-27 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement.

Contrary to what the Examiner has asserted, Applicants did not claim 90% homologs of a homolog. However, to further the prosecution, Applicants have amended the claims to clarify the meaning of the claims.

Naturally occurring proteorhodopsins have known and defined amino acid sequences. Naturally occurring proteorhodopsins have similar amino acid sequences as illustrated by the 81 sequences in Figure 3. Figure 3 also shows the amino acid sequence alignment of 81 naturally occurring proteorhodopsins and the conserved histidine residue. It is not possible for Applicant to list the amino acid sequences of all naturally occurring proteorhodopsins and show the alignments of the sequences to demonstrate the conserved histidine. However, **Applicant has provided a large representative number (81) of species.**

A proteorhodopsin homolog having at least 90% identity with a naturally occurring proteorhodopsin can be made by changing several amino acids of a naturally occurring proteorhodopsin, and testing its ability for undergoing a photocycle containing an "M-state" or "M-like state" (see Paragraph [0108]). This can be easily done by a person skilled in the art.

Therefore, 35 U.S.C. §112, first paragraph of Claims 1, 4-5, 7-9, 14, 23-27 should be withdrawn.

35 U.S.C. 102(e) Rejection

Claims 1, 25-27 remain rejected under 35 U.S.C. §102(e) as allegedly being anticipated by La Rosa et al (US2007/0192889).

La Rosa et al. disclose a plant protein that only shows 7.6% sequence identity with the entire sequence of SEQ ID NO: 3, and 22.4% sequence similarity in a short stretch. When the sequence of the La Rosa protein is aligned and compared with the 81 naturally occurring proteorhodopsins in Figure 3, it is clear that the La Rosa protein is **not** a proteorhodopsin variant comprising a mutation in a conserved histidine residue, wherein said proteorhodopsin variant is a naturally occurring proteorhodopsin or a proteorhodopsin homolog having at least 90% identity with the naturally occurring proteorhodopsin.

Therefore, the 102(e) rejection of Claims 1, and 25-27 over La Rosa et al should be withdrawn.

CONCLUSION

Applicants believe that the application is now in good and proper condition for allowance. Early notification of allowance is earnestly solicited.

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Respectfully submitted,

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PalE7	TMGKLLLLILG	SAIALPSFAA	AGGD....LD	ISDTVGVSFW	LVTAGMLAAT
RED19	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
RED2	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
RED23	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
RED27	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
RED30	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
RED4	.MGKLLLRLG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
RED5	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDA9	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDB9	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDF9	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDr6a5a14	.MGKLLLLILG	SVIALPTFAA	GGGD....PD	ASDYTGVSFW	LVTAALLAST
REDr6a5a6	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDr7_1_15	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASGYTGVSFW	LVTAALLAST
REDr7_1_16	.MGKRLVILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDr7_1_4	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDs3_15	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
REDs3_7	.MGKLLLLILG	SVIALPTFAA	GGGD....LD	ASDYTGVSFW	LVTAALLAST
ANT32C12	..MKLLLILG	SAIALPSFAA	AGGD....LD	ISDTVGVSFW	LVTAGMLAAT
HOT2C02	....	....MKVLML	NPGD......	...HVAISFW	LISMAMVAAT

insert

	51			H	100
BAC31A8	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
BAC40E8	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
BAC64A5	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
HOT0m1	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
HOT75m1	VFFFVERDQV	SAKWKTSLAV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
HOT75m3	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
HOT75m4	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
HOT75m8	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
MB0m1	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB0m2	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB100m10	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB100m5	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB100m7	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB100m9	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB20m12	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB20m2	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB20m5	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB40m1	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB40m12	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MB40m5	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MED101	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MED102	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGSSP....
MED106	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MED202	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MED204	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGSSP....
MED208	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MED25	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGSSP....
MED26	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MED27	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
MED36	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGSSP....
medA15_r8_1	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWV	ETGETP....
medA15_R8_3	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
medA15_r8ex7	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
medA15_R8ex9	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
medA15_r9_3	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
medA15r10b5	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
medA15r11b3	VFFFIERDRV	AAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWV	ETGESP....
medA15r11b9	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
medA15r8b3	VFFFIERDRV	AAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWV	ETGESP....
medA15r8b8	VFFIVERDRV	SSKWKTSLTV	SALVTLIAAV	HYFYMRDVWV	ATGESP....
medA15r8b9	VFFFIERDRV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWV	DSWTGP.GTG

Figure 3-2

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insert

				H	
medA15r8ex4	VFFIVERDRV	SSKWKTSLTV	SALVTLIAAV	HYFYMRDVWV	ATGESP....
medA15r8ex6	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
medA15r9b5	VFFFVERDRV	AAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWV	ETGESP....
medA15r9b7	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
medA17_r8_11	VFFIVERDRV	SAKWKTSLTV	SALVTLIAAV	HYFYMRDVWV	ATGESP....
medA17_r8_15	VFFIVERDRV	SAKWKTSLTV	SALMTLIAAV	HYFYMRDVWV	ATGESP....
medA17_R8_6	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWV	ETGDSP....
medA17R9_1	VFFFIERDRV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWV	DSWNPETGMG
medA19_R8_16	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGGSP....
medA19_R8_19	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGGSP....
medA19_R8_20	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
medA19_r9_9	VFFFVERDQV	SAKWKTSLTV	SGLVTGIAFW	HYLYMRGVWI	ETGETP....
PalB1	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalB2	VFFFVERDQV	SAEWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalB5	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalB6	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalB7	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalB8	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalE1	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalE6	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
PalE7	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
RED19	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
RED2	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
RED23	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
RED27	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
RED30	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGSSP....
RED4	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
RED5	VFFFVERDRV	SAKWKTSLAV	SGLITGIAFW	HYMYMRGVWI	ETGDSP....
REDA9	VFFFVERDRV	SAKWKTSLAV	SGLITGIAFW	HCMYMRGVWI	ETGDSP....
REDB9	VFSFVERDRV	SAKWKTSLTV	SGLITGIAFW	HYMYMRGVWI	ETGDSP....
REDF9	VFFFVERDRV	SAKWKTSLTV	SGLITGIAFW	HYMYMRGVWI	ETGDSP....
REDr6a5a14	VFFFVERDRV	SAEWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
REDr6a5a6	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
REDr7_1_15	VFFFVERDRV	SAKWKTSLTV	PGLITDIAFW	HYMYMRGVWI	ETGDSP....
REDr7_1_16	VFFFVERDRV	SAKWKTSLTV	SGLVTGIAFW	HYMYMRGVWI	ETGDSP....
REDr7_1_4	VFFFVERDRV	SAKWKTSLTV	PGLITDIAFW	HYMYMRGVWI	ETGDSP....
REDs3_15	VFFFVERDRV	SAKWKTSLTV	PGLVTGIAFW	HYMYMRGVWI	ETGDSP....
REDs3_7	VFFFVERDRV	SAKWKTSLTV	PGLITDIAFW	HYMYMRGVWI	ETGDSP....
ANT32C12	VFFFVERDQV	SAKWKTSLTV	SGLITGIAFW	HYLYMRGVWI	DTGDTP....
HOT2C02	AFFFLERDRV	AAKWKTSLTV	AGLVTGIAAW	HYFYMRGVWV	ATGDSP....

	101				150
BAC31A8	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
BAC40E8	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAAGLFKKL	LVGSLVMLVF
BAC64A5	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
HOT0m1	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAAGLFKKL	LVGSLVMLVF
HOT75m1	...TVFRYID	WLLTVPLQMV	EFYLILAACT	SVAASLFKKL	LAGSLVMLGA
HOT75m3	...TVFRYID	WLLTVPLQMV	EFYLILAACT	SVAASLFKKL	LAGSLVMLGA
HOT75m4	...TVFRYID	WLLTVPLQVV	EFYLILAACT	SVAASLFKKL	LAGSLVMLGA
HOT75m8	...TVFRYID	WLLTVPLQMV	EFYLILAACT	NVAASLFKKL	LAGSLVMLGA
MB0m1	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAAGLFKKL	LVGSLVMLVF
MB0m2	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAAGLFKKL	LVGSLVMLVF
MB100m10	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
MB100m5	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
MB100m7	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
MB100m9	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
MB20m12	...TVFRYID	WLLTVPLLIC	EFYLILAAAA	NVAGSLFKKL	LVGSLVMLVF
MB20m2	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAAGLFKKL	LVGSLVMLVF
MB20m5	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
MB40m1	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
MB40m12	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF
MB40m5	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAAGLFKKL	LVGSLVMLVF
MED101	...TVFRYID	WLLTVPLLIC	EFYLILAAAT	NVAGSLFKKL	LVGSLVMLVF

Figure 3-3